TRANSFORMATION *IN VIVO* OF A LARGE RACE OF *ENTAMOEBA HISTOLYTICA* INTO A SMALL RACE*

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WENYON and O'Connor first stated in 1917 that *Entamoeba histolytica* consists of different races distinguishable by the size of their cysts.[1] Dobell and Jepps confirmed these observations the following year.[2] It has been generally believed that the size range of *E. histolytica* strains is a fixed characteristic.

However, Meleney and Zuckerman reported a small race of *E. histolytica* which, after continuing in its small size for 187 days in culture, changed to a large size.[3] The authors exclude the possibility that the observed change could be due to contamination of the culture with another strain of *E. histolytica* because this change was observed independently in two different laboratories situated in two different cities. Further, the small strain originally present in both cultures disappeared after the transformation occurred. What weakens this observation is that 10 years previously Frye and Meleney published a paper in which they stated that while the absence of rice flour from the culture of a large race of *E. histolytica* produced amebae of a smaller size, a small race of *E. histolytica* could not be changed by environmental conditions. On the other hand, this observation was made on a single small strain.[4]

In our unpublished *in vitro* studies of small and large races of *E. histolytica*, the size of the amebae of both races depended on many factors, such as the pH of the media, the degree of anaerobiasis, the addition of such growth-promoting factors as extracts of bacteria or, on the contrary, growth-inhibiting antibiotics. Review of these studies is

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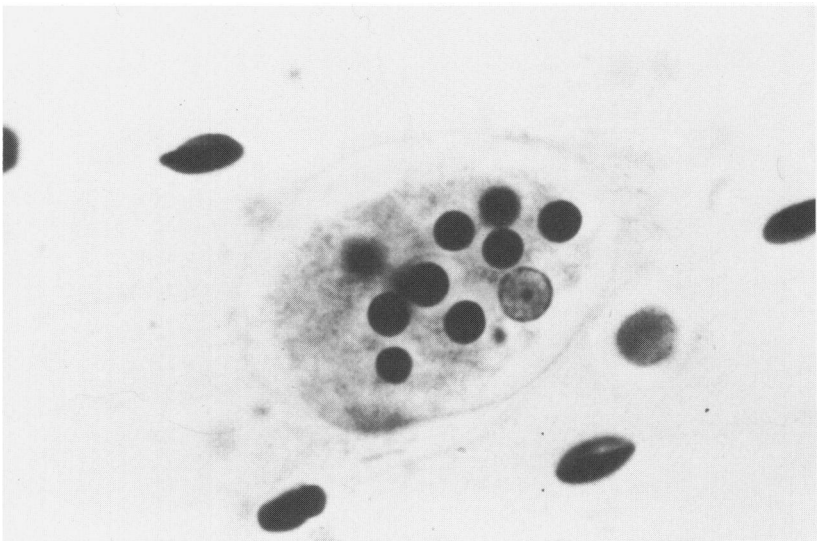

Fig. 1. This trophozoite of *Entamoeba histolytica* was found in the blood-streaked stool of the patient during the initial period of the dysentery. During this period only such large amebae could be found, all having a mean diameter of 41.7 μ. The ameba demonstrates a hyaline peripheral ectoplasm separated from a finely granular endoplasm, and contains numerous dark-stained red blood cells and one diagnostic nucleus with its minute central karyosome and a ring of uniform, peripheral chromatin (below, right). Iron hematoxylin. × 1,000.

beyond the scope of this report, but the influence of antibiotics on the reduction in size of *E. histolytica* in our *in vitro* studies seems to be related to the *in vivo* observations presented today, and is therefore emphasized.

A more striking transformation from large to small *E. histolytica* occurred in a patient with severe amebic dysentery, under observation for a period of 18 months.

The patient, a 40-year-old black man who had served for two years on a U.S. government ship which made regular trips to the Canal Zone, had a bout of diarrhea lasting only a few days. Four years later, in New York, he begun to suffer from severe gastrointestinal distress, with cramps in the lower abdomen, nausea, anorexia, weakness, and 8 to 10 liquid stools a day, followed by rapid weight loss. Upon admission to the New York Veterans Administration Hospital, his blood-streaked stools revealed numerous trophozoites of *E. histolytica*, all very large, having a mean diameter of 41.7 μ, and containing ingested red blood cells (Figure 1).

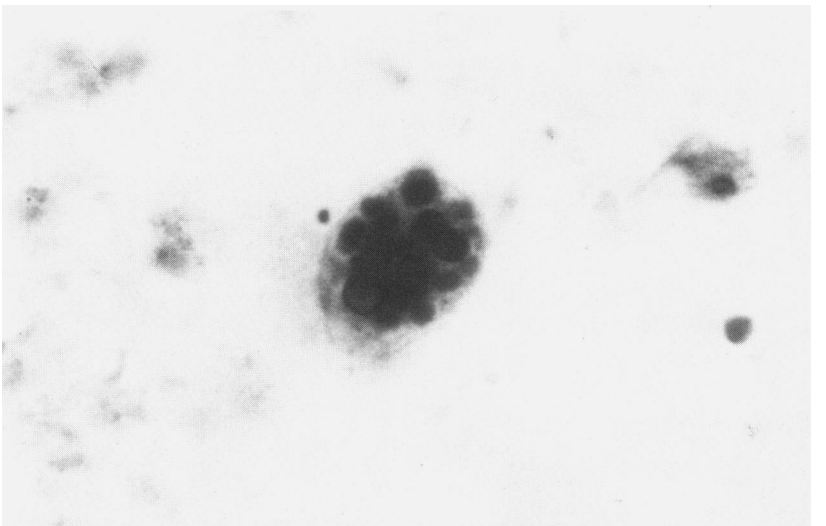

Fig. 2. This trophozoite of *Entamoeba histolytica* was found in the patient's stool during an exacerbation of his intestinal amebiasis while he was receiving bacitracin treatment. The ameba demonstrates ingested red blood cells. The characteristic nucleus is visible (below, left). All of the amebae at this time had a mean diameter of 22 μ. Iron hematoxylin. × 1,000.

A proctoscopic examination showed a granular, hemorrhagic, and diffusely ulcerated mucosa, with areas of focal necrosis and a large amount of exudate. The micro-Kolmer amebic complement fixation test was strongly reactive.

The patient was first given oral bacitracin, starting with 20,000 units, and increasing to 100,000 units per day. In addition, he was given 50,000 units per day rectally. After receiving the total of 1,425,000 units orally and 800,000 units rectally he showed marked improvement. A gradual change was also observed in the proctoscopic appearance of the rectal mucosa, resulting in barely visible areas of erythema and healed ulcerations. The patient passed only one semisolid stool per day, but a few *E. histolytica* could still be found, and the titer of the micro-Kolmer complement fixation test had not decreased below that found on admission. However, the patient was discharged from the hospital and continued treatment on an outpatient basis.

It became obvious at the end of the third week of hospitalization that the size of the trophozoites of *E. histolytica* had gradually decreased, with a diameter never larger than 22 μ. None attaining the original size could

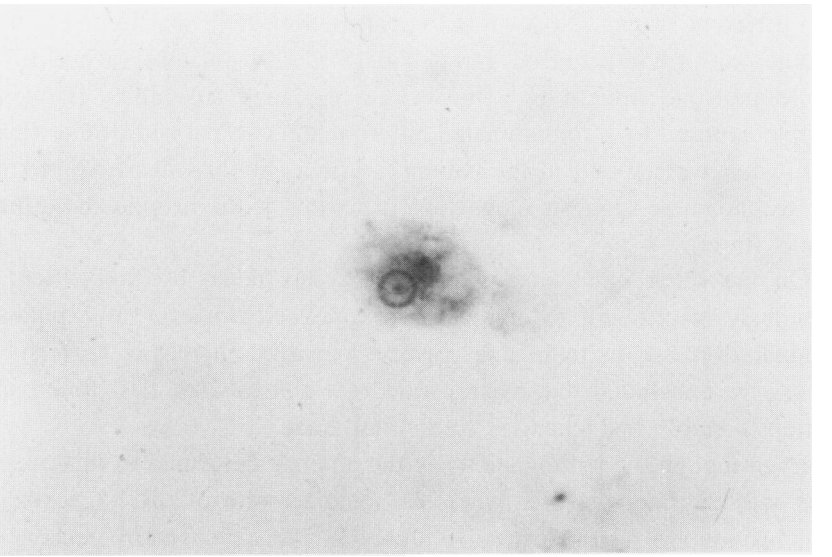

Fig. 3. A trophozoite of *Entamoeba histolytica* found in the patient's stool 18 months after his initial admission to the hospital shows that the amebae further decreased in size, none exceeding 14.3 μ in diameter. The ameba does not contain ingested red blood cells, but has a characteristic *histolytica* nucleus. Iron hematoxylin. × 1,000.

be found, although hundreds of amebae were measured in numerous specimens, even during an exacerbation period when trophozoites of *E. histolytica* contained ingested erythrocytes (Figure 2).

After the patient was given diodoquin, chloroquine, and oxytetracycline, symptoms of the exacerbation period disappeared completely, and the patient passed only one formed stool per day, with no detectable amebae. The patient continued to report to the hospital for laboratory examinations, but not as often as scheduled. On one occasion he returned with diarrhea, and his stools revealed *E. histolytica*, which had further decreased in size. The patient continued to receive treatment, and his stools revealed only rare amebae.

During the patient's last visit to the hospital, 18 months after his first visit, he admitted that he had not taken the drugs as often as prescribed. He still harbored motile trophozoites of *E. histolytica*, the largest of which measured 14.3 μ in diameter (Figure 3), in his stools.

Because amebae harbored by the patient had begun gradually to decrease in size even before he had left the hospital and after hundreds of the amebae had been found and measured, he was not thought to harbor

two different strains. Nor was it logical to conjecture that reinfection with another strain had occurred during the 18 months of observation. In fact, while multiple infections with various parasites are rather frequent in people exposed to a contaminated environment, no parasites other than *E. histolytica* were found in the patient. Further, gradual decrease in size of the trophozoites suggests slow transformation of the original large pathogenic strain.

On the other hand, the quantity of drugs taken by the patient was obviously insufficient to eradicate the amebae. However, it explains the gradual decrease in their size, as also occurred in our *in vitro* studies. Thus, we concluded that a large race of *E. histolytica* first found in the patient's stools had changed to a small race.

Even though the pathogenicity of the amebae described in this presentation was definitely greater when the amebae were of the large size, our conclusions are restricted to size alone. In fact, even many decades ago, when there was much confusion regarding the characteristics of *E. histolytica*, many investigators recognized that the pathogenicity of the amebae varied with different hosts. Thus, Walker and Sellards noticed in experimental studies on human subjects in the Philippines great differences in the responses of individual hosts to infections with the same strain of *E. histolytica*. Thus, the same strain produced amebic dysentery in some subjects, but many others became healthy carriers.[5]

Many factors can influence the virulence of *E. histolytica*. Porter found that a diet rich in protein minimizes the severity of amebiasis, while one high in carbohydrates enhances it. Recognizing the hazard of oversimplification, the author adds that the small cyst-producing type of *E. histolytica* is virtually nonpathogenic, while the large cyst type varies greatly in virulence.[6]

Even a temporary change in diet may influence the pathogenicity of a strain of *E. histolytica*. Kagy and Faust showed that dogs fed raw liver have chronic amebiasis with the production of cysts and experience fulminating dysentery when their diet is changed to canned salmon.[7]

Our conclusions regarding the decrease of a large strain of *E. histolytica* to a smaller size are based on careful measurements, and can be explained by the inhibitory action of antibiotics. Experiments were not carried out to correlate the decrease in size to the decrease in virulence.

SUMMARY

Transformation of a large race of *E. histolytica* into a small race in a patient with acute amebiasis over 18 months of observation has confirmed our belief, born out of previous *in vitro* studies, that the size of the amebic race is not a fixed characteristic, but that environmental conditions can transform a large race of *E. histolytica* into a small one.

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